Collegiate Athlete Runners Study CARS:

Visceral Adipose Tissue and Cortisol in Female Endurance Runners

Undergraduate Research Distinction

By

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Abstract

OBJECTIVE: The relationship between visceral adipose tissue (VAT) and cortisol is a controversial area of research interest. The purpose of this study is to explore this relationship over a training year in an athletic cohort of female endurance runners.

METHODS: Female distance runners (juniors, seniors, or red-shirt seniors) from colleges and universities within a 90-minute drive from The Ohio State University and post-collegiate runners aged 20-26 years living in Columbus, OH who were actively training for a race (>35 miles per week) were eligible for study participation. Laboratory visits occurred three times throughout this longitudinal study. Dietary intake and physical activity questionnaires, an iDXA scan, and an optional blood draw were completed at each visit. Data was analyzed using SAS software (version 9.0) Proc Glimmix and SPSS 27 software. Significance was set at an apriori level of $p < 0.05$.

RESULTS: Eight participants were included in statistical analysis. The average participant was 22.5 years old, 162.56 cm tall, weighed 54.58 kg, and had a BMI of 20.70. No statistically significant relationship was observed between VAT and cortisol ($p = 0.4779$), nor did training mileage, dietary fueling, or energy availability reach statistical significance as potential covariates.

CONCLUSION: No statistically significant relationship exists between VAT and serum cortisol over a one-year training period in this cohort of female endurance runners.

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Table of Content

List of Tables

List of Figures

Chapter 1: Introduction

Though scientific research has made significant advances in the last twenty years, the exact relationship between adipose tissue and chronic disease remains to be elucidated. It has long been known that increased levels of adiposity are associated with negative health outcomes,[1,2] but it is only within the last few decades that body fat has been recognized as a metabolically active, hormonally sensitive tissue rather than a benign site for lipid storage. Though this has led to many paths of scientific inquiry, one that is gaining attention in scholarly literature is the relationship between adipose tissue and stress.[3] The human stress response is largely involved in homeostatic regulation, and it is known to moderate many body systems including metabolism, reproduction, immunity, and cardiovascular function. Hormonally, the stress response is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. This regulatory pathway begins in the brain, and, after stimulation of the adrenal glands, produces the stress-related hormone cortisol.

Of particular interest as of late has been the purported relationship between cortisol and visceral adipose tissue (VAT).[4] This type of fat, associated with internal organs, is associated with greater disease rates than other fat and is increasingly used as an indication of disease risk.[5] Several studies and meta-analyses suggest dysregulation of the HPA axis may be associated with visceral obesity,[3,6] though it is unknown whether this link is a product of the obese state or rather a comorbidity. Therefore, it is obvious that identification of HPA axis dysregulation and cortisol secretion in the development of obesity is a complex discussion. Further complicating this discussion is the consideration of physical activity as both a catabolic, lipolytic state and a stressor that activates the HPA axis,[7] as well as the impact dietary intake may have on HPA axis regulation. Both physical activity and dietary intake are known to have acute impacts on the

HPA axis;[3,7] however, research is lacking concerning the effects of long-term physical activity and dietary intake patterns on HPA axis regulation, cortisol release, and visceral adiposity, especially in athletic populations. The present study, conducted with young adult female distance runners, seeks to answer the question of (1) whether a relationship exists between changes in visceral adiposity and serum cortisol levels over a one-year period of endurance exercise, and (2) if training mileage, dietary intake, and/or energy availability impact this relationship.

Research Question:

> **Does a relationship exist between snapshots of visceral adiposity and serum cortisol levels over a one-year period of endurance training, and if this relationship may be impacted by (or related to) training mileage, dietary fueling, and/or energy availability?**

Chapter 2: Literature Review

The Hypothalamic -Pituitary-Adrenal Axis

A discussion of human cortisol production begins by considering the activity of the hypothalamic-pituitary-adrenal (HPA) axis. This neuroendocrine pathway provides for the stress response during prolonged stimuli, which it accomplishes through the production and sequential release of three hormones: corticotropin-releasing hormone (CRH) from the hypothalamus, adrenocorticotropin hormone (ACTH) from the pituitary, and cortisol from the adrenal glands.[3] Cortisol's release into the blood stream alters many pathways involved in homeostatic regulation, including cognition, reproduction, digestion, and immunity, which together bring about the body's response to stress.[8] Generally, this induces a state where energy stores are mobilized to maximize cognitive and physical performance, and bodily functions not associated with immediate survival experience a temporary cessation.

The centrality of the HPA axis and its role in homeostatic regulation has made it the target of research in both manifest and subclinical disease. Cortisol is considered one of the principle stress hormones involved in this pathway, and therefore it is commonly analyzed in clinical research as an outcome variable of HPA axis activity. Resting cortisol levels are used to indicate basal activity, while cortisol levels following exposure to physiologic or psychologic stressors reflect HPA axis activity during the stress response.[9] Additionally, though the HPA axis is sensitive to regulation by many hormones, cortisol is the hormone most extensively involved in negative feedback and regulation of the axis. This feedback occurs at many places in the brain and peripheral tissues, but the hypothalamus and hippocampus have been implicated as premier feedback sites[8] in addition to the pituitary and adrenal glands. Thus, the measure of cortisol in

the clinical setting is one approach by which to study HPA axis dysregulation and its contribution to adverse metabolic processes.

When discussing HPA axis dysregulation, it is important to recognize how individual genetic differences as well as transient environmental conditions may impact the observed stress response. In a 2007 review, Nieuwenhuizen and Rutters suggest that differences in the stress reaction may result in part from genetic variation that exists at multiple stages of the HPA pathway. They propose ten polymorphisms of the axis, over half of which impact cortisol secretion or sensitivity, and the majority of which have been associated with obesity.[3] When considering testing conditions, Vreeburg et al. conducted a large epidemiological study that sought to analyze how study design itself could impact salivary cortisol measures. Their study design sought to tightly control the variability of inter-subject testing conditions, but they found statistically significant differences in cortisol awakening response (CAR) and diurnal slope between subjects who tested on workdays, had differences in time of waking on testing day, and the amount of daylight in a given month.[9] Interestingly, the amount of daylight impacted more cortisol markers than the other study sampling factors, exhibiting a negative correlation with total cortisol and a slower decline throughout the day.[9] Though further research is needed concerning individual variations in HPA axis functioning, sampling factors, and cortisol levels, these studies highlight the complex nature of genetic variation and study design variability that underline any discussion of metabolic processes.

Cortisol in Humans

Although cortisol release of the adrenal glands is largely mediated by individual physiological and psychological conditions, there are aspects of cortisol production and function

that are conserved in humans. Healthy individuals exhibit a diurnal cortisol secretory pattern marked by elevated morning levels, low evening levels, and variable rates of decrease throughout the day.[3,5] The internal and external stimuli, real or perceived, that regulate cortisol release are thought to do so in an additive manner to the basal levels observed in the diurnal slope.[5] Following cortisol's release into vascular circulation, corticosteroid-binding globulin (CBG) functions as the chief protein involved in the hormone's transport (binds approximately 90% of circulating cortisol), though cortisol also binds albumin with lower affinity.[3,10] While much of the circulating cortisol is bound, a small fraction is not, and thus is biologically active. As well, a small amount of the cortisol precursor, cortisone, is also available for responsive conversion to cortisol by the enzyme 11-betahydroxysteroid dehydrogenase type I (11HSD1).[10] In fact, over expression of 11HSD1 has been implicated as a target of cortisol mediated inflammation. The body's regulation of this intricate hormonal web is peripheral as well as central.

At peripheral tissues (such as visceral adipose tissue), cortisol is acted upon by two bidirectional 11B-hydroxysteroid dehydrogenases. In vivo, type I (11HSD1) generally uses reductase activity to regenerate cortisol from cortisone, while the type II (11HSDII) facilitates the reverse dehydrogenase reaction[3,10]. A study conducted with eight healthy men (defined in part as BMI 22.7 ± 0.5 kg/m^2, no chronic illnesses reported) found that cortisol concentration increased faster in hepatic vein blood (peak reached at 76 min.) than in arterialized blood (peak reach at 118 min.) following oral cortisone administration, suggesting that the liver regenerates cortisol more rapidly than peripheral tissues.[11] However, the same study predicted that extrahepatic splanchnic tissues, including visceral adipose tissue, may contribute up to two-thirds of total splanchnic cortisol regenerated in non-adrenocorticotropic hormone-dependent

production (hepatic regeneration rate =15.2 nmol/min, VAT rate = 29.8 nmol/min).[11] A study analyzing cortisol regeneration in obese subjects showed that women with abdominal body fat distribution (android) had a higher urinary cortisone:cortisol ratio, suggesting greater peripheral metabolism of cortisol in the kidneys.[12] In lay terms, these studies suggest that the majority of cortisol regeneration may occur in tissues other than the liver when the HPA axis is in an unstressed state.

Cortisol and Visceral Adipose Tissue (VAT)

Currently, scientific literature supports the involvement of two classes of cellular receptors in cortisol metabolism. These are mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), both of which act as ligand-activated transcription factors.[13] MR receptors are generally thought to be bound during basal HPA axis activity, whereas GR receptors are implicated in binding activity with high cortisol concentrations that are typically observed following adrenal stimulation of the stress response.[13] Interestingly, when looking at specific tissue responses, VAT has the highest glucocorticoid receptor concentration of any type of fat.[4] This may be significant when considering data that suggests glucocorticoids stimulate adipocyte differentiation and maturation,[3,10] implying a possible correlation between elevated cortisol levels and greater levels of visceral adiposity. Abdominal fat deposition, especially visceral adipose tissue, is one of the greatest risk factors of chronic disease,[4] so research analyzing the relationship between VAT and cortisol may provide insight into the multifactorial etiology of obesity and its related diseases.

A unique relationship between cortisol and visceral adipose tissue is supported by further research. Cortisol awakening response (CAR) was measured in lean, obese, and reduced obese (defined as recent or current weight loss of greater than 5 kg) subjects to examine the influence of body fat distribution on cortisol secretion. No change was found between lean men and lean women, but weight distribution and weight loss showed variable effects on cortisol levels in obese subjects.[14] In looking at 165 overweight, minority youth, cortisol was positively associated with visceral adipose tissue but not total body adipose measures such as weight, BMI, total body fat, and waist circumference. Additionally, no association was reported between cortisol and subcutaneous adipose tissue (SAT), suggesting a specificity in cortisol metabolism that depends on the type or location of adipose tissue.[4] These studies support the theory that body fat distribution, specifically levels of visceral adiposity, are pertinent to a discussion of HPA axis dysregulation and individual differences in cortisol secretion.

Furthermore, the relationship between cortisol and VAT in the development of chronic disease may be the product of tissue-specific regulation of cortisol metabolism, as regulated by cortisol receptors and 11HSDs. A 2001 study found that men with the highest BMI had a statistically significant increase ($p< 0.01$) in total urinary cortisol metabolites, but this association was no longer significant when adjusting for lean body mass. Additionally, the study found no difference in total plasma cortisol following a CRH stimulation test.[15] Similar results were observed in a cohort of 41 Caucasian women: though a positive trend emerged between higher ACTH release and greater BMI ($P = 0.06$), the values remained insignificant.[16] These results suggest that total cortisol metabolism and/or circulating cortisol levels were not significantly elevated in obese subjects. Notably, however, in vitro fat biopsy analysis showed greater 11HSD1 activity in the adipose tissue of obese subjects,[15] which was again reported in the

adipose tissue of obese women based on correlation analyses of fat biopsies and tetrahydrocortisol to tetrahydrocortisone urinary ratios.[16] This supports the assertion that the type, location, and stimulation frequency of 11HSD enzymes and cortisol receptors may mediate cortisol's effect independent of total cortisol levels.[13] The apparent link between visceral adipose tissue and peripheral cortisol metabolism suggests that cortisol receptors and hydrogenase enzymes may work together to mediate cortisol's tissue specific responses.

Cortisol, VAT, and Chronic Disease

In the absence of diagnosed disease known to directly involve the adrenal glands, such as Cushing Syndrome and Addison's Disease, the question regarding cortisol and visceral adipose tissue in the development of chronic disease is two-fold: (1) whether there is an association between cortisol and chronic disease and (2) if scientific evidence favors a comorbid correlation or a causative relationship. When considering the link between cortisol and VAT, obesity becomes a germane chronic disease. In the health care setting, some obese patients present with many clinical features of hypercortisolism, such as glucose intolerance, dyslipidemia, and a central distribution of body fat.[3,5,17] Moreover, the phenomena known as thin outside, fat inside (TOFI), is used in describing individuals with seemingly normal weight but disproportionate amounts of visceral adipose tissue. This suggests that characterizing the relationship between cortisol and adipose tissue is important for thin individuals as well as obese who could experience adverse health effects and may be at increased risk of developing chronic disease.

Although the interplay between the HPA axis and diseases of the reproductive system are beyond the scope of this particular review, hormones associated with female menstruation must be considered when analyzing HPA axis regulation as they have been suggested to impact its

activity. Interestingly, in a 2009 epidemiological study, Vreeburg et al. found no significant impact on cortisol secretion when analyzing the presence of menstruation, phase in the menstrual cycle, or oral contraceptive use as potential covariates.[9] Additionally, much of the current scientific literature involving female subjects is conducted during the follicular phase of the menstrual cycle, and the use of oral contraceptives is either an exclusion factor or accounted for in statistical analysis. It is likely that menstrual hormones are not a confounding variable in studies examining the relationship between HPA axis regulation and cortisol secretion.

Greater variability in cortisol levels between awakening and before bed have been associated with greater sensitivity of the HPA axis to feedback inhibition by dexamethasone,[18] suggesting that regulation of the HPA axis may be more important than total levels of cortisol in relation to chronic disease. Dexamethasone, a synthetic glucocorticoid agonist, is used to measure sensitivity of the HPA axis to negative feedback by suppressing endogenous cortisol and ACTH production.[5] When analyzing research using the dexamethasone suppression test, retained sensitivity of the pituitary in obesity is both supported and challenged by research. Dexamethasone suppression tests including 53 premenopausal, obese women did not show a direct effect on cortisol.[12] In a study of obese male subjects, total plasma cortisol was actually significantly lower ($P< 0.01$) following dexamethasone suppression and subsequent oral cortisone administration.[15] These studies suggest that the pituitary may remain sensitive to glucocorticoid feedback inhibition with obesity. In contrast, a dexamethasone suppression test administered to patients diagnosed with metabolic syndrome showed statistically significant higher concentrations of ACTH when compared to controls,[17] suggesting patients with metabolic syndrome are less sensitive to the negative feedback mechanisms involved in cortisol regulation by the HPA axis.

Alterations of the HPA axis were also seen in 50 premenopausal, obese women kept in a tightly controlled environment and subject to standardized timing of meals and activities.[6] Women with abdominal body fat distribution (A-BFD) showed higher salivary cortisol response and peak measurement following a dexamethasone suppression test, which may reflect a hyperresponsiveness of the HPA axis.[6] A-BFD participants also had greater overnight cortisol secretion compared to those with peripheral body fat distribution (P-BFD), but no statistical difference was reported for 24-hour total cortisol secretion.[6] The results of this study suggest that the relationship between HPA axis dysregulation and obesity may be evident when considering body fat distribution, which would not be surprising considering the proposed relationship between cortisol and VAT. The contradictory evidence of these studies does not dismiss the involvement of HPA axis dysregulation in obesity, but rather it may showcase variations in fat distribution (central/android obesity vs. peripheral/gynoid obesity) that further complicate obesity's multifactorial etiology.

Exercise as a Stress State

The communication between adipose tissue and HPA cortisol regulation likely has many mediators, and exercise should be considered as one such covariate. Not only does physical activity (PA) stimulate the HPA axis, therefore making it a stressor,[7] but the effects of PA on adiposity are well documented. In one example, Wong et al. (2004) found that lower total abdominal fat was correlated with higher levels of cardiovascular fitness (reported as VO_2 max; measured by maximal treadmill testing) at a given BMI in male subjects,[19] highlighting the correlation between fitness and decreased central adiposity. The catabolic effect of exercise is also seen specifically on visceral adiposity. In the same study, men with higher cardiovascular

fitness had significantly decreased amounts of visceral adipose tissue compared to less fit subjects ($p < 0.001$).[19] In fact, this association was seen even though a statistical significance between cardiovascular fitness and BMI was not observed. In another study, a cohort of 70-year-old obese men showed a significant decrease in visceral adipose tissue following a 10-week exercise intervention.[20] Interestingly, meta-analyses have suggested that, though exercise amount and intensity approach a dose-dependent response on VAT decrease, the relationship itself remains insignificant.[21] It is interesting then to consider how exercise, as a catabolic, cortisol-releasing state is related to basal cortisol levels, and if the relationship exists in a dose-dependent manner.

Physical activity is known to have acute effects on the HPA axis, but the exact impact that PA has on circulating cortisol levels in the short-term is still unknown. In examining PA's immediate impact on cortisol levels, it was found that non-athletic female participants who walked 30 minutes prior to engaging in a psychological stress test (Trier Social Stress Test for Groups, TSST- G) had lower total salivary cortisol secretion than those in a non-walking intervention group.[7] This relationship existed independent of prior fitness level,[7] suggesting that the difference in cortisol secretion was due to study participation in acute exercise rather than chronic. However, in another study that measured cortisol immediately following exercise, a study population of 53 obese women demonstrated no difference in salivary cortisol levels,[12] though the research team hypothesized the exercise may have mitigated lunch-time cortisol response. Therefore, scientific evidence attempting to explain the acute impact of physical activity on circulating cortisol levels is contradictory.

Similarly, scientific literature examining the relationship between habitual exercise and chronic cortisol levels is also contradictory. Though much research exists suggesting a

physiological adaptation to the chronic, repeated stress of exercise, just as many studies exist discrediting this hypothesis. Wood et al. reported that females with low heart rates following 30 minutes of moderate-intensity exercise (defined as ≤ 70% maximal heart rate, 141 bpm) had significantly lower total cortisol secretion compared to those with high heart rates (> 141 bpm),[7] suggesting that prior training and fitness levels may regulate cortisol levels. The participant's average heart rate during walking accounted for approximately nine percent of the observed variance in total cortisol secretion (measured as area under the curve with respect to ground [AUCg]) in hierarchical multiple regression analysis.[7] Likewise, in examining health factors thought to modify cortisol secretion, increased physical activity was reported to correlate with greater cortisol secretion upon awakening (measured as CAR) followed by a steeper decline in cortisol concentrations during the day when compared to individuals who self-reported less PA.[9] The effect of PA on these two cortisol measures had the highest statistical significance of all analyzed factors. Individuals who self-reported more PA also showed greater cortisol suppression following dexamethasone administration,[9] possibly conveying increased sensitivity of the HPA axis. In contrast, a study of 44 women, split evenly into higher and lower fitness levels by VO_2 max parameters, found no statistical significance between plasma cortisol levels following the consumption of a mixed meal (61% carb, 20% protein, 19% fat).[22] Considering that study designs examining the correlation between a snapshot of physical fitness and cortisol levels have produced contradictory data, this secondary analysis seeks to examine the statistical relationship (or lack thereof) between cortisol levels and variable exercise intensity from multiple snapshots taken over a year period.

When discussing exercise intensity and cortisol, another idea for consideration is overtraining, which is typically considered as an inability to fully recover from exercise sessions.

In a study with six elite weightlifters, no statistical correlation was found between calculated fatigue index (mathematical model based on training intensity and training response) and serum cortisol levels over a one-year period. Furthermore, the correlation coefficients for each individual varied, with half showing a negative correlation and half showing a positive one between the two variables.[23] In a study at The University of Wisconsin-Madison, 14 female varsity swimmers showed no significant increase in baseline salivary cortisol levels taken during periods of regular exercise and serum samples taken during a three-week period of overtraining (defined as ~ 12,000 yards/day).[24] However, in three swimmers identified as having a performance decrease between 5-10% of normal capacity, statistically significant increases were observed in salivary cortisol compared to teammates who had not experienced performance reductions.[24] Similarly, in a study of female and male rowers, there was no significant difference in subjects' serum cortisol levels between periods of variable exercise intensity over a seven-week training period. In subgroup analysis, one male rower who almost collapsed during competition had the highest levels of cortisol of all participants four days prior to the event.[25] He also had the highest serum urea concentration of all study participants, which the researchers suggested may indicate greater protein degradation and possible overtraining.[25] However, the changes in measured serum markers failed to reach significance for this individual. Though no association was observed between training intensity and cortisol levels in those athletes with sustained capacity for athletic performance, the association between cortisol levels in athletes with marked decreases in performance may warrant further scientific investigation.

Cortisol and Dietary Intake

It is known that acute stress decreases the activity of digestive processes and suppresses appetite, while cortisol's release mobilizes energy stores by inducing the catabolism of glycogen,

lipid, and protein. Cortisol along with its CRH precursor are proposed to exert this influence on dietary intake and macronutrient metabolism by acting on the central biological processes that regulate food intake.[3] The hypothalamus is sensitive to regulation by hormones associated with feeding [leptin and insulin] in addition to cortisol, and the hypothalamus also produces hormones that influence dietary intake such as neuropeptide Y and agouti-related peptide.[8] However, this secondary analysis seeks to examine the reverse question: does there appear to be a correlation between serum cortisol levels and a snapshot of dietary intake? Studies addressing this question are limited. Due to the HPA axis's regulatory nature, it is important to approach this discussion considering the impact of long-term dietary patterns on cortisol levels.

When considering the impact that lipid consumption patterns may have on cortisol, many studies have chosen to analyze lipid in the subcategories of saturated, monounsaturated, and polyunsaturated; a few studies even considered specific fatty acid compounds. In their study of adult Mediterranean women, García-Prieto et al. found a statistically significant difference in the fatty acid profiles of women with high and medium HPA axis sensitivity (measured by dexamethasone suppression test) compared to those with an attenuated response. Total saturated fatty acid (SFA) intake was significantly elevated in the low sensitivity group, specifically myristic, stearic, and palmitoleic acids when analyzed individually, while monounsaturated fatty acid (MUFA) intake was significantly decreased.[18] This suggests that lipid intake favoring saturated fatty acids rather than unsaturated fatty acids may contribute to decreased sensitivity of the HPA axis. In the same study, a statistically significant inverse relationship was also seen between dexamethasone suppression and overall fat intake, where greater intake of total fat correlated with less HPA axis sensitivity ($p = 0.045$). In looking at the dietary intake of 165 overweight Hispanic and African American youth, Gyllenhammer et al. examined omega-3 fatty

acid intake and found that only youth with omega-3 intake below the study mean showed a positive correlation between VAT and plasma cortisol.[4] No significance was reported between total fat intake and cortisol. Though these studies conflict on whether total fat intake has an impact on HPA axis regulation and cortisol secretion, they both suggest that fat intake favoring saturated fats rather than unsaturated may have a mediating influence on the relationship between HPA axis regulation, cortisol, and visceral adipose tissue.

Similarly, when considering the long-term intake of carbohydrates, the type of carbohydrate consumed may be more important than total carbohydrate consumption. In addition to their analysis of omega-3 fatty acid intake as a potential mediator between VAT and cortisol, Gyllenhammer et al. observed that high dietary sugar intake (defined as total sugar consumption exceeding the study population mean [22.7% of total kcal] and/or ≥ 10% total kcal from added sugars) showed a positive correlation between serum cortisol and VAT in regression analysis.[4] However, there was no association observed between VAT and cortisol when considering total carbohydrate.[4] In another study considering 41 adult women living in Murcia, a town on the Mediterranean coast of Spain, the percentage of total kilocalories from carbohydrate was positively and significantly correlated with greater suppression of endogenous cortisol production, and therefore greater HPA axis sensitivity, following dexamethasone administration.[18] Though the study did not analyze macronutrient subcategories to differentiate between sugar, starch, and fiber, it aids the previous study in suggesting that sugar, but not carbohydrate generally, may be a modifying factor in the relationship between cortisol and visceral adipose tissue.

Studies examining the impact of dietary intake on cortisol levels in athletic populations are very limited, likely due to training intensity and schedule demand. In one study by Mielgo-

Ayuso et al., the idea that dietary quality rather than total macronutrient intake may modify HPA axis regulation is again seen in an athletic population, but the impact of dietary intake on cortisol levels is questionable. Twenty-two elite volleyball players from the Spanish First National Professional League were recruited to participate in the study during their 29-week competitive season, during which every athlete completed the same training program consisting of approximately 30 minutes of aerobic exercise, 90 minutes of strength training, and 3 hours of game-technique practice daily (match and rest days excluded). A statistically significant inverse association was found between carbohydrate intake (measured in g/kg/day) and levels of ACTH ($r = -0.658$).[26] Interestingly however, no significant association was found between specific macronutrient intake and changes in serum cortisol over the competitive season.[26] This supports the conclusion found in general populations that greater total carbohydrate intake was associated with the regulation and sensitivity of the HPA axis, but no association was reported between dietary intake and cortisol levels in athletic populations.

However, there are quite a few important aspects to note about the previous study that highlight the need for more scientific research concerning the relationship between dietary intake and cortisol. The study only included general adiposity measures (skinfold measurements) and did not consider these measurements as confounding variables in the statistical analysis, which, as in the previous study with minority youth, may elucidate the relationship between adiposity, cortisol, and HPA axis function. Additionally, the study showed that total carbohydrate intake was significantly and continuously lower than the recommended intake for elite athletes in this population, an outcome that was again observed in a study considering dietary intake patterns in professional female tennis players (measured in both 7-dietary records and 24-hour recall performed over a 29-week competitive season).[26,27] This may have an additive effect in

explaining the lack of association observed between adiposity and cortisol in athletic populations, as adequate and/or excessive carbohydrate may be a modifying factor between cortisol and VAT.[4,18] Finally, it should be noted that the study length exceeded half a year, but this may not have been sufficient time to observe changes in resting cortisol from diet and/or exercise. This suggests that alterations in HPA axis regulation due to diet may require repeat, chronic exposure lasting greater than six months to occur to an appreciable extent. The current secondary analysis seeks to address these issues by considering body composition, dietary intake, and approximately one year of study with three measures over the course of the study.

Closing Summary

Though this literature review is far from comprehensive, it explored the relationship between cortisol and visceral adipose tissue while considering exercise and dietary intake as potential mediating factors. A unique relationship was proposed between cortisol and VAT, namely that VAT has a higher cortisol receptor concentration than other types of fat,[4] and that cortisol promotes adipocyte differentiation and maturation.[3,10] This relationship may be regulated by the activity of 11HDS enzymes and cortisol receptors in a tissue-specific manner.[15,16] When considering the impact of cortisol and visceral adiposity on chronic disease, it was suggested that the relationship between HPA axis dysregulation and obesity may be evident when considering body fat distribution, specifically central/android obesity vs. peripheral/gynoid obesity.[6] When considering how exercise impacts cortisol and VAT, there is greater statistical support for an attenuating effect of exercise on VAT than an effect of exercise on cortisol levels.[19,20] Studies relating basal cortisol levels to habitual exercise are contradictory, and further research concerning cortisol and overtraining may help to elucidate this relationship.[24] Finally,

when considering dietary intake, studies tend to point to dietary quality, such as the intake of complex carbohydrates and unsaturated fatty acids, as having a potential mediating effect on cortisol levels.[4,18]

Chapter 3: Methods

1. Subjects

Subjects were recruited for study participation by flyer and word of mouth. Informational flyers were sent to head cross-country coaches as well as head athletic trainers of all colleges and universities within a 90-minute drive from The Ohio State University. Due to the limited interest and/or ability to participate of collegiate female cross-country runners, study participation was opened to former collegiate distance runners living in Columbus, OH. Inclusion criteria for students included upperclassman status (junior, senior, or red-shirt senior), while inclusion criteria for post-collegiate distance runners included actively training for a race, running more than 35 miles per week during their heaviest training period, and being between 20-26 years of age. Subjects were excluded from study if an underclassman or pregnant at the time of laboratory visits (health risk associated with radiation of iDXA scans). Eleven women were recruited to partake in this longitudinal study, but three were excluded in this secondary analysis due to inadequate serum cortisol data. The study was approved by the Institutional Review Board (IRB protocol number 2018H0248), and participants were required to sign the Consent/HIPAA and iDXA intake forms prior to participation.

2. Lab Visit

Laboratory visits occurred three times throughout this longitudinal study, with the goal of pre-season (August), peak-season (November), and post-season (May) visits. Lab visits for collegiate runners more closely followed this timeline than did the lab visits for post-collegiate runners; lab visits for post-collegiate runners occurred roughly during January, May, and

October/November/December. During or before each visit, subjects completed a pre-testing questionnaire that included dietary intake assessment using the Vioscreen Food Frequency Questionnaire (FFQ) and a 3-day (2 weekdays, one weekend day) food record analyzed with ESHA food processor (version 11.6.522) as well as estimated physical activity for the previous three months using the Modifiable Activity Questionnaire (MAQ). Pace and mileage data were collected as standard interview questions during each patient's iDXA scans. Training volume was then calculated by multiplying mileage times the inverse of pace, and energy availability was calculated by subtracting calories expended from calories consumed and dividing by the individual's lean body mass (LBM). On the day of their laboratory visit, subjects were asked to fast 3-4 hours prior to the morning visit, abstain from alcohol consumption for 12 hours prior, avoid vigorous activity for 12 hours prior, and dress in appropriate athletic attire.

2.1 iDXA

The laboratory protocol included calibration of the iDXA machine prior to its use on each day of laboratory visits. Each lab visit included a full set of iDXA scans including the total-body scan, thus body composition was provided as a 3-compartment model. The CoreScan software is included on the GE Lunar iDXA (enCORE software version 15 SP4) and estimated the amount of visceral adiposity (VAT) for each subject at each visit.

2.2 Lab blood draw and analysis

For participants who opted to partake in the optional blood draw, approximately 25-35 ml of blood was drawn by a trained phlebotomist at each laboratory visit. Samples were refrigerated

and delivered to the hospital laboratory for analysis of cortisol among other analytes. Some of the serum was aliquoted and samples were stored in -80-degree freezer for later analysis of some of the inflammatory markers.

3. Statistical Analysis

SAS software (version 9.0) was used to develop a mixed general linear regression model using Proc Glimmix. Proc Glimmix was chosen as it allows each subject to have her own intercept and analyze her unique change in serum cortisol level across time (slope). This is an advanced regression method, which allows for the control of variables such as training mileage and nutritional markers during analysis. Additionally, the Proc Glimmix model is especially useful as it allows for the inclusion of study participants with incomplete data sets (specifically cortisol in this study) in statistical analysis. Descriptive data was analyzed using SPSS 27 software and was used to characterized participants both at entry and at each visit. Significance was set at an apriori level of $p < 0.05$.

Chapter 4: Results

Eight female runners from the study were included in data analysis. At the study's onset, the average participant was 22.5 years old, 162.56 cm tall, weighed 54.58 kg, and had a BMI of 20.70. The age range of participants was 20 – 25 years, as study participation was open to competitive post-graduate runners. Notably, one runner met the criteria for underweight BMI classification (BMI < 18.5 kg/m^2), while the remaining seven participants fell within the normal BMI classification (18.5 kg/m^2 to 24.9 kg/m^2). Participant's descriptive data at baseline is included in Table 1.

Table 1. Participant Characteristics at Baseline

Anthropometrics	Mean	Standard Deviation	Minimum	Maximum
Age, y	22.50	± 1.85	20.00	25.00
Height, cm	162.56	± 5.282	152.40	168.90
Weight, kg	54.68	± 4.8561	45.60	61.10
BMI, kg/m^2	20.70	± 1.9131	17.80	24.00

When looking at participant characteristics by visit, average plasma cortisol was lowest among participants at visit 2 (15.591 mcg/dL) and peaked at visit 3 (19.729 mcg/dL). Similarly, average participant mileage followed the same pattern, with its lowest recorded value of 35.00 miles/week at visit 2 and rising to 39.67 miles/week at visit 3. Conversely, average VAT mass decreased at each visit, starting at 0.209 kg at visit 1 and ending at 0.150 kg on visit 3. Average pace increased at each visit, and training volume varied due to individual differences in

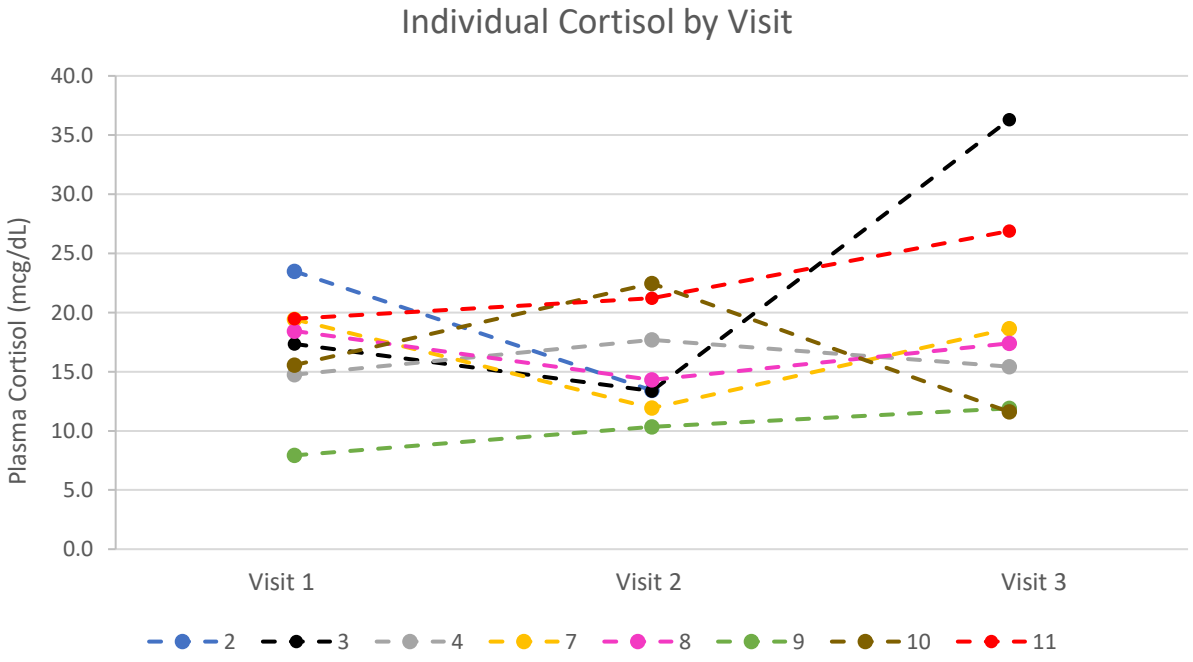
competition preparation. These data are shown in Table 2. It is important to note that these data are snapshots of the measured markers and do not represent continuous changes/trends.

Table 2. Participant Characteristics by Visit

	Visit 1	Visit 2	Visit 3
Cortisol, mcg/dL	17.054 (4.563)	15.591 (4.398)	19.729 (8.915)
VAT, kg	0.209 (0.147)	0.163 (0.095)	0.150 (0.090)
Total Body Fat, kg	11.02 (2.43)	10.98 (2.79)	11.93 (3.11)
Mileage, miles/week	38.50 (11.43)	35.00 (16.33)	39.67 (20.66)
Pace, minutes/mile	7.813 (0.704)	7.833 (0.931)	8.083 (0.665)
Training Volume, mileage*pace^{-1}	5.022 (1.719)	3.791 (0.957)	4.354 (2.337)

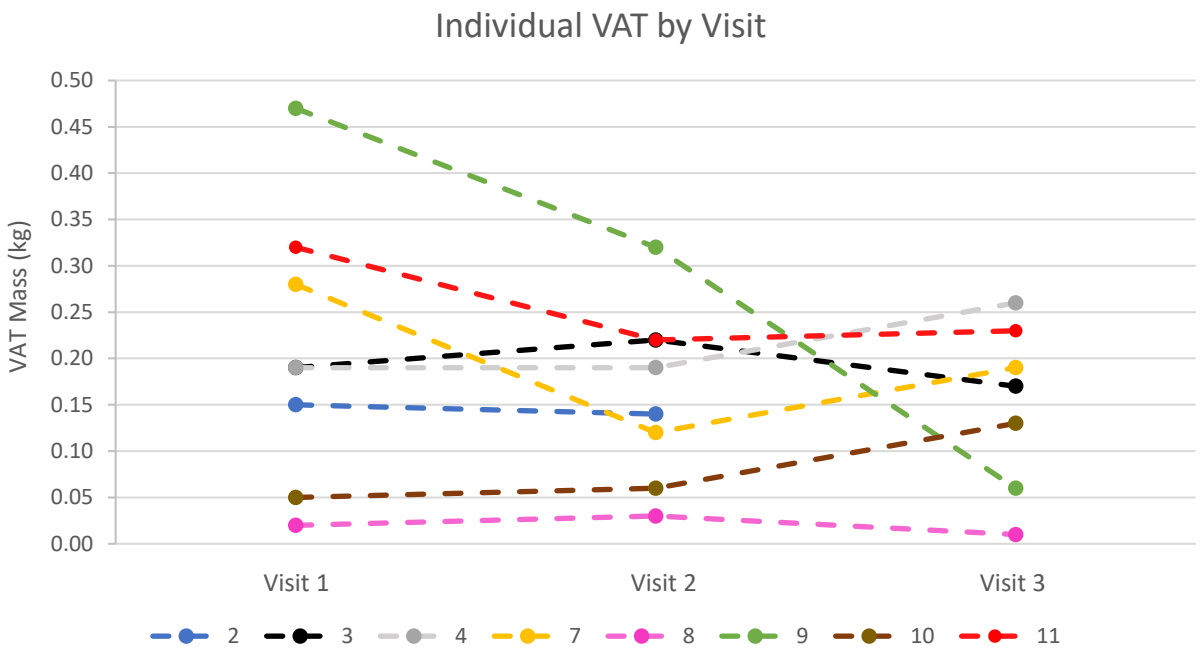
*Values reported as mean (SD)

Figure 1. Participant Plasma Cortisol by Visit



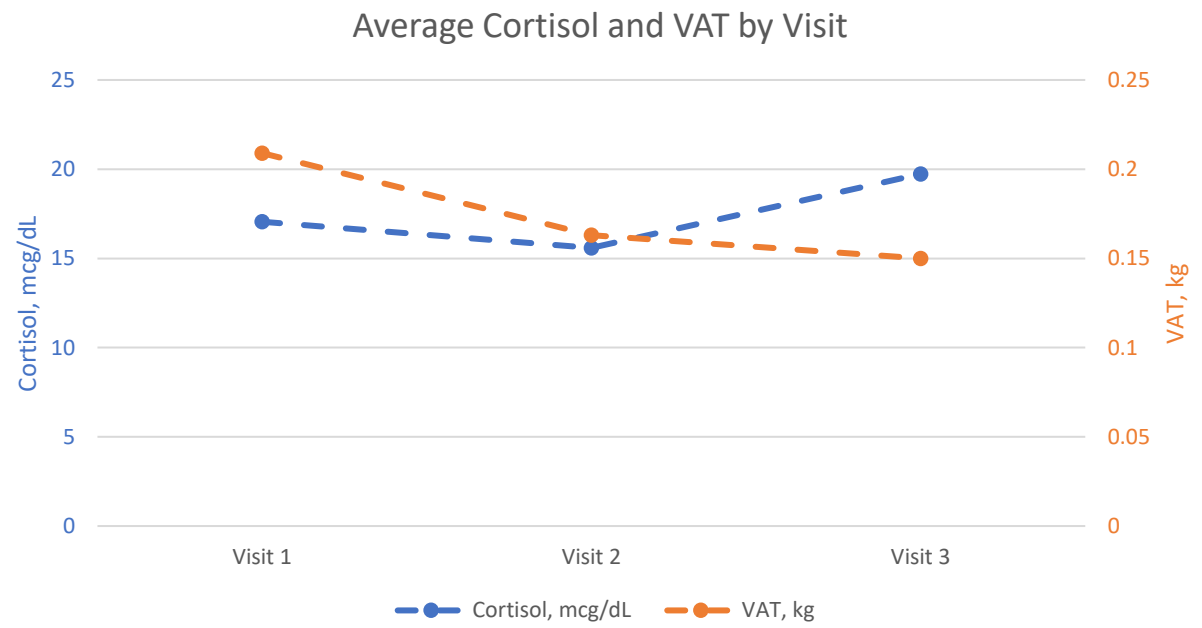
*Lines do not represent gradual change over time and are only included to visualize trends in individual values.

Figure 2. Participant VAT Mass by Visit



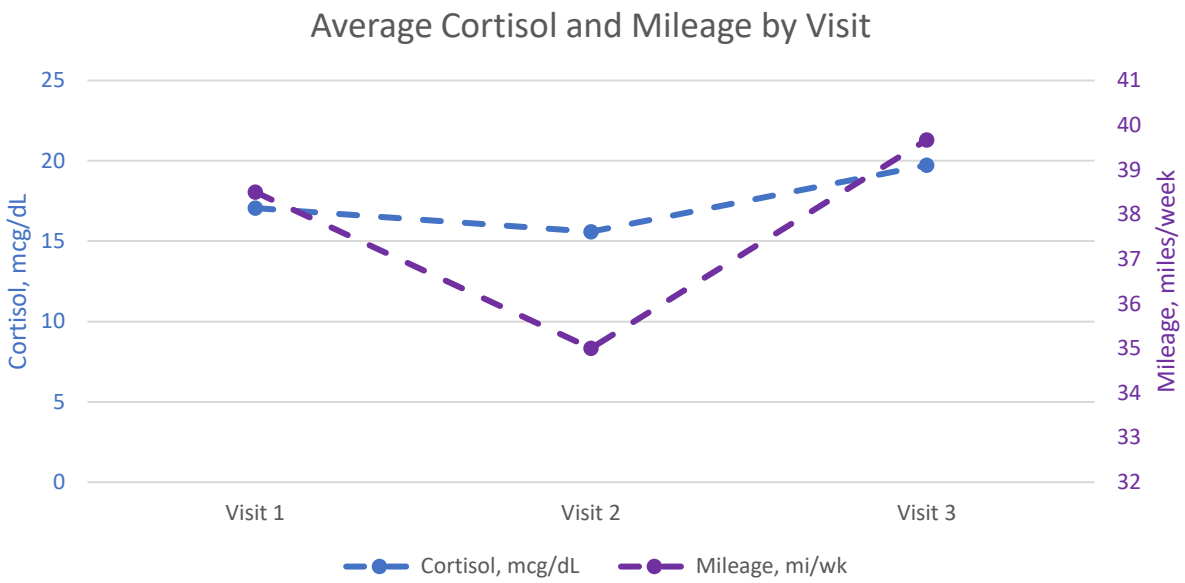
*Lines do not represent gradual change over time and are only included to visualize trends in individual values.

Figure 3. Average Cortisol and VAT by Visit



*Lines do not represent gradual change over time and are only included to visualize trends in individual values.

Figure 4. Average Cortisol and Mileage by Visit



*Lines do not represent gradual change over time and are only included to visualize trends in individual values.

VAT and Cortisol

The relationship between VAT and cortisol was nonsignificant in bivariate or higher multivariate analyses. Analyzing VAT mass in relation to cortisol alone produced a p-value of 0.4779 (Table 4), while multivariate analysis controlling for visit produced a larger p-value of 0.5737 (Table 5). Additionally, visit was analyzed as a potential contributing variable for both VAT and cortisol to ensure variance in individual testing dates did not impact the study outcomes. No significant relationship was found between visit and either VAT or cortisol. (Table 3).

Table 3. The Impact of Visit on VAT and Cortisol

The Impact of Visit on VAT and Cortisol				
Dependent Variable	Type III Test P-value	Visit 1	Visit 2	Visit 3
VAT	0.3719	0.1897	0.7280	.
Cortisol	0.3793	0.3665	0.1756	.

Training Mileage, Dietary Fueling, and Energy Availability

When considering potential covariates, no statistically significant relationships were observed between VAT, cortisol, and any of the other variables of interest (energy availability, dietary fueling, or training mileage). In bivariate analysis, the relationship between VAT and EA produced the lowest p-value ($p = 0.0710$), but this number still failed to reach statistical significance (Table 4). Dietary fueling was considered by analyzing caloric and glucose intakes with p-values of $p = 0.0939$ and $p = 0.5271$ respectively. When the same variables were analyzed controlling for visit, the p-values increased (Table 5). Training mileage was considered along with pace and training volume to provide a more comprehensive view of training status. The relationship between these variables and VAT was analyzed controlling for visit and cortisol, and all resulting p-values were greater than $p = 0.1500$ (Table 6).

Table 4. VAT Response to Cortisol, EA, Calories, and Glucose

Impact of Independent Variables on VAT				
Dependent Variable	Cortisol		EA	
VAT	Type III Test P-value	Estimate	Type III Test P-value	Estimate
	0.4779	-0.00261	0.0710	0.005004
	Glucose		Calories	
	Type III Test P-value	Estimate	Type III Test P-value	Estimate
	0.5271	0.001247	0.0939	0.000123

Table 5. VAT Response to Cortisol, EA, Calories, and Glucose when Controlling for Visit

Impact of Independent Variables on VAT Controlling for Visit							
Dependent Variable	Type III Test p-Value				Visit 1	Visit 2	Visit 3
VAT	Cortisol	0.5735	Visit	0.4223	0.2591	0.8977	.
	EA	0.1500	Visit	0.7740	0.6160	0.9246	.
	Calories	0.2188	Visit	0.8520	0.6231	0.9475	.
	Glucose	0.9794	Visit	0.5859	0.3773	0.8761	.

Table 6. VAT Response to Mileage, Pace, and Training Volume when Controlling for Visit and Cortisol

Impact of Independent Variables Controlling for Visit and Cortisol						
Dependent Variable	Type III Test p-Value					
VAT	Mileage	0.1522	Visit	0.4608	Cortisol	0.3294
	Pace	0.6464	Visit	0.6000	Cortisol	0.6008
	Training Volume	0.6423	Visit	0.6457	Cortisol	0.5771

Chapter 5: Discussion

The primary objective of this study was to determine if a relationship exists between snapshots of visceral adiposity and serum cortisol over a one-year period of endurance training. The relationship between VAT and cortisol was nonsignificant in linear regression analysis ($p \geq 0.4779$), and remained nonsignificant when controlling for dietary fueling, energy availability, and training mileage as potential covariates.

Existing research detailing the relationship between VAT and cortisol is limited in athletes. Published research typically explores the relationship of cortisol to overweight and obesity, which has produced inconsistent, diverse results: both increased and decreased responses of the HPA axis and cortisol production have been recorded with overweight and obesity.[12,14–17] However, general measures of adiposity such as weight, BMI, waist circumference, and total body fat are often used in this literature, which may not be appropriate considering cortisol's specificity for VAT[4,14] and may help explain the lack of consistency. Studies using BMI or total body fat as a proxy measure cannot adequately be compared to the present study, and, because cortisol has not been well evaluated next to VAT, few studies were available for comparison. Of the studies included in this paper's literature review, no studies with lean or athletic individuals included VAT as a measured value.[14,16,23–26] Of the two studies that observe the relationship between adiposity and cortisol in non-overweight and non-obese subjects, no data was found suggesting a dysregulation of cortisol in this population.[14,15] Studies concentrating on athletes only considered cortisol's response to either exercise training or dietary intake and did not include markers of body fatness in analysis.[23–26]

In considering VAT specifically, an observational study by Gyllenhammer et al. showed a positive association between cortisol and VAT in 165 overweight, minority youth.[4] This contrasts

the results of the present study. However, endurance runners included in the present study had an average VAT mass of 0.209 ± 0.147 kg at baseline, whereas youth in the Gyllenhammer et al. study had an average VAT mass of 1.563 ± 1.012 kg,[4] an average that is 7x greater (Table 7). Additionally, the lowest average VAT mass of the present study was recorded at visit 3 (0.147 ± 0.101), and the greatest individual VAT mass measured was 0.47 kg (visit 1). Although the population in Gyllenhammer et al. is nonathletic African American and Hispanic youth and cannot be directly compared to the present study, it is tempting to consider that total levels of visceral adiposity and body fatness may be important in elucidating the relationship between cortisol and visceral adipose tissue. This idea is supported by the minimal VAT mass in the present study's population, as well as previous studies with lean subjects that showed no statistical significance between levels of body fatness and cortisol.[14,16] However, it must be noted that subgroup analysis in Gyllenhammer et al. showed the association of cortisol and VAT only remained significant in subjects with high total and added sugar intakes.[4] This suggests then that diet must be considered as a potential covariate, and maybe even more so in obesity. More research is needed in both general and athletic populations to define this relationship.

Table 7. VAT and Total Body Fat

Levels of VAT in Gyllenhammer and the Present Study				
	Gyllenhammer		*Present Study*	
	Mean (SD)	Range	Mean (SD)	Range
Total Body Fat (kg)	35.4 (12.6)	67.8	11.0 (2.4)	7.4
VAT (kg)	1.563 ± 1.012	5.242	0.209 ± 0.147	0.450

Metabolically, athletes rely on blood glucose and muscle glycogen to fuel their exercise, and carbohydrate ingestion, especially post-exercise, is critical to maintaining energy stores for future exercise. This increased carbohydrate use during exercise increases overall carbohydrate

needs compared to inactive individuals. Still, Hinderer previously reported that only one of the eight subjects included in the present analysis met the recommended carbohydrate intake threshold of 8 g/kg/day for peak performance at their activity level, and three of the eight subjects met the criteria for inadequate EA (< 30 kcal/kg fat free mass).[28] Inadequate carbohydrate intake was also seen in professional female tennis players[27] and elite volleyball players from the Spanish First National Professional League.[26] These volleyball players showed no statistically significant association between carbohydrate intake and serum cortisol over a 29-week competitive season.[26] Though the results of Gyllenhammer et al. implicate dietary intake as a modifying factor between cortisol and VAT, this may not be a confounding variable in athletic populations. Instead, this relationship may be impacted by metabolic demands and intake patterns (especially in female athletes) that differ from the general population.

In looking at runners specifically, Jennewine reported that less than half of 100 premenopausal female runners were consuming adequate carbohydrates for their activity level as defined by the International Olympic Committee (6-10 g/kg/d for 1^{+} hour of endurance exercise), and the average study participant had an EA of 32.6 (± 12.61).[29] In subgroup analysis, a significant increase was reported in mean serum cortisol of runners with adequate carbohydrate intake compared to those with inadequate intake, but no relationship was observed when considering carbohydrate intake as a continuous variable.[29] Considering that adequate and/or excessive carbohydrate may be a modifying factor between cortisol and VAT,[4,18] chronic inadequate carbohydrate intake and more generally low EA may help explain the lack of association observed between dietary intake and cortisol in female athletic populations. More research is needed concerning the relationship between carbohydrate intake, cortisol, and VAT, but the results of this study suggest the relationship is nonsignificant in female athletes.

Because training mileage (as well as pace and training volume) data was collected at three time points across a year period, this study lends itself to discussing the impact of chronic, habitual exercise on cortisol and VAT. The results of the present study suggest that training mileage does not impact the relationship between VAT and cortisol. Unfortunately, no studies were found for comparison that consider these three variables together in analysis. The nonsignificant relationship observed between training mileage and cortisol supports the conclusions of a study of 44 women where fitness levels (dependent on VO_2 max) had no impact on serum cortisol, but it should be noted that this study measured cortisol after consumption of a mixed meal.[22] In the present study, each participant's training mileage varied due to the subject's individualized competition dates and training plans, but mileage per week averaged 38.50 at visit 1, fell to 35.00 at visit 2, and peaked at 39.67 for visit 3. The current literature was searched for studies regarding the cortisol response in athletes with higher weekly mileage (i.e. ironman competitors or ultramarathoners), but studies in this population have analyzed the cortisol response during singular day endurance events rather than habitual training. Interestingly, though no runners included in the present study were noted as having decreased performance capacity, published research may suggest a relationship between cortisol and training status in overtrained athletes. No statistical correlation was found between training intensity and cortisol levels when looking at total populations of elite powerlifters, female collegiate varsity swimmers, and collegiate rowers,[23–25] but increased cortisol levels were observed in teammates who experienced marked reductions in athletic performance.[24,25] Data measured in this study does not allow for subgroup analysis for a potential impact of overtraining.

Limitations

Limitations of this study include convenience sampling methods. Because study participants were recruited by flyer, the study sample constitutes a convenience sample, meaning data may not be reflective of female endurance runners generally. Participants ideally would be selected to participate randomly, minimizing selection bias. Additionally, the small sample size (n=8) decreases statistical power, so p-values approximate trends rather than significance. This limitation may be corrected by increasing the number of individuals willing and able to participate. Compounding the impact of the small sample size are the missing values from three subjects in data collection, which suggests a large patient burden associated with scheduling and attending multiple in-person visits over a year period. Finally, the highly variable timing between visits may have impacted data collection, as testing conditions have been shown to impact measured values, specifically cortisol.[9] Due to individual differences in training plans, subject visits did not always represent the intended pre-season and post-season periods of training in parallel with each other. For instance, many study participants trained harder between visits 2 and 3 then they did between visits 1 and 2. This was not the same for all subjects. Future studies would benefit from conducting data collection on standardized days.

Another important limitation occurred with the subjective data collection techniques. Self-reported data is subjective by nature and therefore invites the possibility of inaccuracy, intentional or not. The results of the study may have been strengthened by collecting dietary intake and physical activity information by objective means. Collecting accurate dietary intake records for all eight participants would have been largely infeasible, but physical activity information may have been reported by using standardized mileage and pace trackers (such as digital running watches). Additionally, this would have provided more comprehensive data of

each runner's weekly activity and more accurately reported mileage, pace, and intensity than the MAQ estimates.

Finally, cortisol samples were analyzed at Ohio State's university hospital and the research group was not in possession of them during this time. Samples were placed at the hospital laboratory drop-off window, and the time elapsed between drop-off and processing is unknown. This introduces the possibility that the sample was mishandled, especially during busy intervals. Therefore, the research group would need to retain possession of the samples to be confident they were handled the correct way.

Future Studies

Placing the results of this study in the context of published research was difficult due to the lack of standardized testing methods used. Many of the studies discussed in the literature review used different measures of adiposity as well as different biomarkers to explore the relationship between HPA axis function, cortisol levels, and visceral adiposity. Some studies analyzed cortisol precursors (namely ACTH) to suggest a dysregulation of the HPA without also measuring cortisol. In one study analyzing athlete's dietary intake over a competitive season, a statistically significant relationship was seen with ACTH that was not seen with cortisol.[26] This is certainly an area of research that would benefit from standardized approaches as well as increased research interest. For athletic populations specifically, it may be beneficial to explore the relationship between cortisol and VAT in athletes with relatively high amounts of VAT mass, such as those who participate in strongman competitions, or team sports where body composition is highly variable by position specialization, such as American football.

Conclusion

The results of this study support the null hypothesis: no statistically significant relationship exists between VAT and serum cortisol over the one-year training period in this cohort of female endurance runners. Additionally, no relationship was found between cortisol and VAT with dietary intake, energy availability, nor training mileage. Athletes, specifically female endurance runners, may present a unique population in which to study this question as their VAT levels, dietary intake, and training patterns differ from the general population. Specifically, this population tends to have minimal VAT mass and inadequate intake relative to their activity level. Nevertheless, this area of study is fertile for research in endurance athletes to further delineate the training response to various levels of intensity and mileage in different body types.

References

1. De Lorenzo A, Romano L, Di Renzo L, Di Lorenzo N, Cenname G, Gualtieri P. Obesity: A preventable, treatable, but relapsing disease. *Nutrition*. 2020;71:110615. doi:10.1016/j.nut.2019.110615

2. Bendor CD, Bardugo A, Pinhas-Hamiel O, Afek A, Twig G. Cardiovascular morbidity, diabetes and cancer risk among children and adolescents with severe obesity. *CARDIOVASCULAR DIABETOLOGY*. 2020;19(1). doi:10.1186/s12933-020-01052-1

3. Nieuwenhuizen AG, Rutters F. The hypothalamic-pituitary-adrenal-axis in the regulation of energy balance. *Physiology & Behavior*. 2008;94(2):169-177. doi:10.1016/j.physbeh.2007.12.011

4. Gyllenhammer LE, Weigensberg MJ, Spruijt-Metz D, Allayee H, Goran MI, Davis JN. Modifying influence of dietary sugar in the relationship between cortisol and visceral adipose tissue in minority youth. *Obesity (Silver Spring, Md)*. 2014;22(2):474-481. doi:10.1002/oby.20594

5. Björntorp P, Rosmond R. Obesity and cortisol. *Nutrition*. 2000;16(10):924-936. doi:10.1016/S0899-9007(00)00422-6

6. Duclos M, Gatta B, Corcuff J-B, Rashedi M, Pehourcq F, Roger P. Fat distribution in obese women is associated with subtle alterations of the hypothalamic–pituitary–adrenal axis activity and sensitivity to glucocorticoids. *Clinical Endocrinology*. 2001;55(4):447-454. doi:10.1046/j.1365-2265.2001.01384.x

7. Wood CJ, Clow A, Hucklebridge F, Law R, Smyth N. Physical fitness and prior physical activity are both associated with less cortisol secretion during psychosocial stress. *Anxiety, Stress & Coping*. 2018;31(2):135-145. doi:10.1080/10615806.2017.1390083

8. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci*. 2006;8(4):383-395.

9. Vreeburg SA, Kruijtzer BP, van Pelt J, et al. Associations between sociodemographic, sampling and health factors and various salivary cortisol indicators in a large sample without psychopathology. *Psychoneuroendocrinology*. 2009;34(8):1109-1120. doi:10.1016/j.psyneuen.2009.04.024

10. Gathercole LL, Lavery GG, Morgan SA, et al. 11β-Hydroxysteroid dehydrogenase 1: translational and therapeutic aspects. *Endocr Rev*. 2013;34(4):525-555. doi:10.1210/er.2012-1050

11. Andrew R, Westerbacka J, Wahren J, Yki-Järvinen H, Walker BR. The Contribution of Visceral Adipose Tissue to Splanchnic Cortisol Production in Healthy Humans. *Diabetes*. 2005;54(5):1364-1370. doi:10.2337/diabetes.54.5.1364

12. Duclos M, Pereira PM, Barat P, Gatta B, Roger P. Increased Cortisol Bioavailability, Abdominal Obesity, and the Metabolic Syndrome in Obese Women. *Obesity Research*. 2005;13(7):1157-1166. doi:10.1038/oby.2005.137

13. Sapolsky RM, Romero LM, Munck AU. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. 2000;21(1):35.

14. Therrien F, Drapeau V, Lalonde J, et al. Awakening Cortisol Response in Lean, Obese, and Reduced Obese Individuals: Effect of Gender and Fat Distribution. *Obesity*. 2007;15(2):377-385. doi:10.1038/oby.2007.509

15. Rask E, Olsson T, Söderberg S, et al. Tissue-specific dysregulation of cortisol metabolism in human obesity. *J Clin Endocrinol Metab*. 2001;86(3):1418-1421. doi:10.1210/jcem.86.3.7453

16. Rask E, Walker BR, Söderberg S, et al. Tissue-Specific Changes in Peripheral Cortisol Metabolism in Obese Women: Increased Adipose 11β-Hydroxysteroid Dehydrogenase Type 1 Activity. *J Clin Endocrinol Metab*. 2002;87(7):3330-3336. doi:10.1210/jcem.87.7.8661

17. Kazakou P, Kyriazopoulou V, Michalaki M, Ierodiakonou V, Psyrogiannis A, Habeos I. Activated Hypothalamic Pituitary Adrenal Axis in Patients with Metabolic Syndrome. *Horm Metab Res*. 2012;44(11):839-844. doi:10.1055/s-0032-1311632

18. García-Prieto MD, Tébar FJ, Nicolás F, Larqué E, Zamora S, Garaulet M. Cortisol secretary pattern and glucocorticoid feedback sensitivity in women from a Mediterranean area: relationship with anthropometric characteristics, dietary intake and plasma fatty acid profile. *Clin Endocrinol*. 2007;66(2):185-191. doi:10.1111/j.1365-2265.2006.02705.x

19. Wong SL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Cardiorespiratory Fitness is Associated with Lower Abdominal Fat Independent of Body Mass Index. *Medicine & Science in Sports & Exercise*. 2004;36(2):286-291. doi:10.1249/01.MSS.0000113665.40775.35

20. Ballin M, Lundberg E, Sörlén N, Nordström P, Hult A, Nordström A. Effects of Interval Training on Visceral Adipose Tissue in Centrally Obese 70-Year-Old Individuals: A Randomized Controlled Trial. *Journal of the American Geriatrics Society*. 2019;67(8):1625-1631. doi:10.1111/jgs.15919

21. Ross R, Soni S, Houle S a. Negative energy balance induced by exercise or diet: Effects on visceral adipose tissue and liver fat. *Nutrients*. 2020;12(4). doi:10.3390/nu12040891

22. Jayasinghe SU, Torres SJ, Fraser SF, Turner AI. Cortisol, blood pressure, and heart rate responses to food intake were independent of physical fitness levels in women. *Applied Physiology, Nutrition & Metabolism*. 2015;40(11):1186-1192.

23. Busso T, Häkkinen K, Pakarinen A, et al. A systems model of training responses and its relationship to hormonal responses in elite weight-lifters. *Eur J Appl Physiol*. 1990;61(1):48-54. doi:10.1007/BF00236693

24. O'Connor PJ, Morgan WP, Raglin JS, Barksdale CM, Kalin NH. Mood state and salivary cortisol levels following overtraining in female swimmers. *Psychoneuroendocrinology*. 1989;14(4):303-310. doi:10.1016/0306-4530(89)90032-2

25. Urhausen A, Kullmer T, Kindermann W. A 7-week follow-up study of the behaviour of testosterone and cortisol during the competition period in rowers. *Europ J Appl Physiol*. 1987;56(5):528-533. doi:10.1007/BF00635365

26. Mielgo-Ayuso J, Zourdos MC, Urdampilleta A, Calleja-González J, Seco J, Córdova A. Relationship of long-term macronutrients intake on anabolic-catabolic hormones in female elite volleyball players / Relación de la ingesta a largo plazo de macronutrientes en las hormonas anabólicas-catabólicas de jugadoras de voleibol elite. *Nutrición Hospitalaria*. 2017;34(5):1155-1162. doi:10.20960/nh.763

27. Filaire E, Massart A, Hua J, Le Scanff C. Dietary Intake, Eating Behaviors, and Diurnal Patterns of Salivary Cortisol and Alpha-Amylase Secretion Among Professional Young Adult Female Tennis Players. *International Journal of Sport Nutrition & Exercise Metabolism*. 2015;25(3):233-242.

28. Hinderer A. Collegiate Athlete Runner Study CARS: A Longitudinal Analysis of Dietary Intake in Comparison with Dietary Recommendations for Athletes. Published online 2020. Accessed March 24, 2021. https://etd.ohiolink.edu/apexprod/rws_olink/r/1501/10?clear=10&p10_accession_num=osu1587466845699645

29. Jennewine J. Interrelationship of Diet, Visceral Adipose Tissue and Cortisol in Pre-menopausal Female Runners. Published online 2015:94.